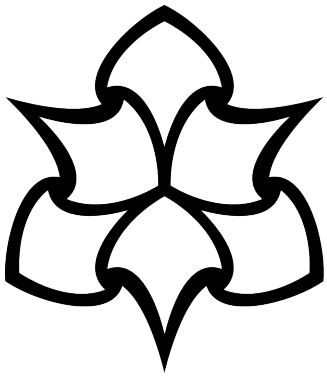

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Optimisation of biogas generation from brown seaweed residues: compositional and geographical parameters affecting the viability of a biorefinery concept.

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Abstract

Very recently, integrated biorefinery approaches are being developed with the aim to produce high-value products for a variety of industries in conjunction with green energy from sustainable biomass. Macroalgae (seaweed) have been regarded as more sustainable compared to terrestrial crops, since they do not occupy land for growth. Macroalgal biomass changes greatly according to species and harvest season, which affects its chemical energy potential. This study was conducted seasonally on five species of brown seaweed over a yearlong period to investigate the effects of chemical composition variations, bioproducts extraction processes and inoculum acclimatation on methane production. As a result of the bioproducts extraction, it was found the seaweed residues exhibit a great potential to produce methane. Stoichiometric methane yield and C:N ratio changed in favour of an improved digestibility with bioconversion rates greater than 70% in some instances, i.e. achieved by *Laminaria* species and on the West coast *Fucus serratus*. The two *Laminaria* species investigated also presented the highest CH_4 production rate, with *Laminaria digitata* reaching 523 mL CH_4 gVS^{-1} and L. *saccharina* peaking at 535 mL CH_4 gVS^{-1} with acclimatised and non-acclimatised sludge respectively.

Keywords: Macroalgae Residues, Extraction, Integrated Biorefinery, Methane Potential, Acclimatation, Anaerobic Biodegradability.

Acronyms: AD (Anaerobic Digestion), BI (Biodegradability Index), COD (Chemical Oxygen Demand), TS (Total Solids), VS (Volatile Solids).

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1. Introduction:

Seaweed biomass has been under the spotlight as feedstock for biogas production in the recent years. Seaweeds (or macroalgae) are regarded as third generation feedstocks for biofuels, since their use as energy crops exhibits several advantages when compared to terrestrial crops [1]. In particular, they are not quite used as food source on a global scale, which minimises the impact on price related to the food versus fuel debate for first generation feedstocks, e.g. corn or palm oil. Furthermore, unlike second generation lignocellulosic crops such as wood, maize or grass, cultivation of macroalgae does not occupy arable land. This translates into multiple benefits for cultivating marine crops. These are low or absent in lignin content (recalcitrant to biofuels conversion), no fresh water or nutrients provision is needed for growth. Also, faster growing rates than land crops and higher CO_2 remediation potential have been reported due to a more efficient photosynthesis [2]. Nonetheless, seaweed conversion to biofuels encounters several technical challenges such as seasonal variation in composition also depending upon geographical location [3] and necessity to undertake assessments of the impact of systematic wild-harvesting on marine ecosystems. Hence, cultivation techniques need to be improved or re-designed to fulfil specific species' requirements locally.

The EU has underpinned that seaweed biogas or biomethane as transport fuel may be playing a significant role for energy generation in the near future [4]. However, the main obstacle to harvesting the seaweed-to-energy potential lies in the vast volumes of biomass required to generate meaningful energy contribution to help the shift towards replacement of fossil fuels. A very interesting study by Allen et al. [5] on seaweed gaseous biofuels suggests its feasibility would be possible if suitable volumes of feedstock are obtained via aquaculture. It is yet unknown how this can be achieved sustainably as aquaculture techniques developed so far are not cost-effective to justify the use of this resource solely for energy purposes. A review by Ghadiryanfar et al. [6], identifies that, despite being more cost-competitive than other renewables, biofuels and bioenergy from macroalgae entail higher costs than terrestrial biomass due to costly cultivation. In their study, in fact, Roesijadi et al. [7] regards this as a key issue. The authors also identify that biogas production from macroalgae is more technically-viable than for other fuels however, the cost of marine crops needs to be reduced by 75% of the present level to make macroalgal biogas economically-feasible. The feasibility of algal biofuels can be significantly enhanced by a high-value co-product strategy [8] using an integrated biorefinery approach to produce simultaneously bioproducts and biofuels to

enable circular economies. A comprehensive review by Jung et al. [9] on potentials of macroalgae as feedstock for biorefinery, reports that macroalgal biomass is currently utilized to source human food, algal hydrocolloids, therapeutic materials, fertilizer, and animal feed. The food industry, whose market share is 83–90% of the total seaweed industry, is the largest and accounts for $5 billion worldwide on an annual basis [7]. This means that the remaining 10-17% used for extraction of bioproducts would be available to explore integrated biorefinery opportunities. In fact, when processed for extraction of bioproducts, a significant amount of sugar-rich seaweed residue is generated, which can easily be used for feeding anaerobic digesters. A study by Tedesco and Stokes [10] has investigated the biogas potential from macroalgal biorefined residues in October harvested in Co. Clare, Ireland. The authors identified a biogas potential between 182 and 453 mL gVS^{-1}, with best results achieved from *Laminaria* spp. although they have not analysed the effect of seasonal variation on such yields.

Seasonal variation in composition has a major influence in determining the methane potential from marine biomass. Therefore, in order to obtain a stable biogas production, investigations are needed over a year period to assess the variability of the methane yields achievable and plan for complementary co-substrates for digestion. Very few studies have been conducted on seasonal composition of macroalgae [11-13]. These, however, have not investigated biorefined algal residues but have rather characterised the biomethane potential from freshly harvested or drift seaweed biomass. Nizami et al. [14] reported that the selection or integration of biorefinery technologies should be based on its waste characterisation. As biochemical characterisation changes seasonally in the fresh feedstock, it is expected that algal residues will also present a changing composition depending upon harvesting periods. Seasonal variation in composition was also found to represent one of the major technical challenges for seaweeds in the biobased economy by van Hal et al. [15].

Brown macroalgae have been selected for this research as these are mainly used in Irish industrial applications across a variety of sectors. Ireland's seaweed-based industry consists of small and medium businesses involved in production of animal nutrition, animal hygiene, plant health, soil fertilizers, alginate, cosmetics and nutraceutical products [16]. The Irish Fishery Board (BIM), reported that the Irish seaweed production and processing industry will be worth €30 million per annum by 2020 [17]. The waste products generated by this growing industry are not currently characterised for biofuels production. The literature heavily lacks of investigations examining the seasonal biogas potential from the algal waste streams

derived from the existing bio-industry. Since feasibility studies on biogas generation from waste solids and liquids from seaweed processing plants are also relevant to government authorities [18], this study aimed at characterising the methane yield response from the most common Irish brown seaweed residues generated by the local bioindustry.

Anaerobic digestion (AD) essays have been conducted over a yearlong period during which brown seaweed biomass has been wild-harvested seasonally at two opposite sides of the island, in order to evaluate the influence of geographical location on composition. These are Howth Bay, Co. Dublin on the East coast and a number of bathing beaches in Co. Galway and Co. Clare on the West coast.

The harvested feedstocks underwent bioproducts extraction using room temperature extraction procedures provided by the project industry partner (Irish Seaweed Processors Ltd.) based in Ireland. The extraction processes used in this research also follow the seaweed biorefinery concept proposed by Balina et al. [19] in which polysaccharides, antioxidants, pigments and proteins are targeted by the extraction cascade that precedes a biogas production step, which utilises the leftover residue as input feed for AD. The biochemical composition (1) was analysed after collection of the biomass and again following bioproducts extraction to identify the residual organics content. Effective methane yields (2) and biodegradability indices (3) against the theoretical stoichiometric yields were used to evaluate the methanogenic potential against the actual methane yields from the feedstocks. As the resulting pH of suspended residues solution was highly alkaline, acclimatation (4) of the inoculum for improved gas yields was also tested and yields compared with performance of non-acclimatised inoculum.

2. Materials and Methods:

2.1. Substrate collection and inocula

Biomass of *Fucus serratus* (FS), *Fucus vesiculosus* (FV), *Ascophyllum nodosum* (AN), *Laminaria digitata* (LD), and *Laminaria saccharina* (LS) was collected seasonally at low tide (2015-16) and underwent extraction at room temperature of bio-compounds at laboratory scale, as per procedure provided by the industry partner (Irish Seaweed Processors Ltd). The extracting procedure adopted by the processing company targets the extraction of alginic acid, fucoidan, fucoxantin, laminarin, mannitol, and proteins.

The collections took place on the East and West coasts of Ireland in order to investigate the effect of geographical location on the biomass composition. The harvesting sites were Co. Galway and Co. Clare beaches on the West side of the island with collections in May, September, November and January, and Howth Bay on the East side with collections in June, October, November and January. Samples were harvested and frozen within 24 hours to -20°C until use. The collections started in May/June 2015 and were completed in January of the following year.

In order to add the necessary fermenting microorganisms to the reactors, the residue samples were then incubated with 300 g of digested sewage sludge, provided by the wastewater treatment plant of Celtic Anglian Water (CAW) Ltd. The initial sludge's pH was measured as 8.1±0.02. Acclimatation was conducted by inoculating reactors with extracted residue of the same seaweed species to be subsequently digested and allowing fermentation to occur for approximately 10 days before incubation in the reactors.

2.2. Proximate and ultimate analysis

Dry organic matter or Total Solids (TS) and Volatile Solids (VS) contents were determined by using a high-temperature oven via overnight drying of the samples at 105 °C, followed by combustion at 575°C of the seaweed residues, as by standard procedure [20]. Tests were conducted in duplicate.

The ultimate analysis was outsourced to Celignis Ltd. (Irish biomass laboratory) to identify the elemental composition of the fresh and residual substrates. The carbon, hydrogen, nitrogen, and sulphur contents of samples were obtained according to the European Standard procedure EN 15104:2011 [21], using an Elementar Vario MACRO Cube elemental analyser. The oxygen content was calculated by difference according to the formula in eq. 1:

Oxygen (%) = 100 - Carbon(% Dry Basis) - Hydrogen(% Dry Basis) - Nitrogen(% Dry Basis) -Sulphur(% Dry Basis) - Ash(% Dry Basis) (eq. 1)

2.3. Bioproducts extraction methods

Approximately 200 g of each individual macroalgal species' fronds were manually chopped down to roughly <0.5cm and sealed in a food plastic bag, which was then extensively perforated to maximise soaking in the reagent solution. Bags were kept below solvent level

by the aid of a weight. To simulate the industrial scale extraction process, the biomass species were extracted together in series using three separate buckets respectively containing 3L of ethanol 99.9% pure, then a mild acid (acetic acid pH 5.5) and finally a 5L solution of 10% w/w Na_2CO_3 (pH 9.5) at room temperature for the duration of 3 hours per extraction step. Ambient temperature was selected for the extractions as it has been proven to be almost as efficient as high-temperature extractions [22], thus constituting a cheaper alternative for seaweed processors to obtain bioproducts. Samples were then manually squeezed for about a minute and dried at 105±2 °C overnight in a muffle furnace. They were then cooled down and stored in a desiccator until use. The proximate and ultimate analysis on the extracted residues was determined by the methods described in the previous section.

2.4 pH measuring and adjustments

The samples' pH was measured using a Hanna precision pH meter, model pH 213 prior to and after digestion was completed. Furthermore, pH adjustments were required as following the last alkaline extraction with 10% w/w Na_2CO_3, pH of the residues was found above 9. As this value is not suitable for a well performing AD process, which has been found to be 7.5 – 8.5 [23, 24], adjustments were carried out with 0.1N sulphuric acid solution in order to bring the initial pH to neutral.

2.5. Batch experiments

The bioreactors set-up was conducted following procedure VDI 4630 [25]. The reactors consisted of borosilicate glass flasks of 500 ml each in capacity. Each bioreactor was filled with 300 g of inoculum (digested sewage sludge or acclimatised sludge) and 20 g of seaweed residues. Each bioreactor condition was performed in triplicate. The pH of each sample was adjusted to neutral prior to incubation with the inoculum. A biogas analyser, model Drager X-Am 3000, was used to verify anaerobic conditions were created correctly when preparing the reactors and to analyse the gas composition at the end of the collection period. An upturned measuring cylinder was utilized to derive the biogas volume. The whole system configuration is replicated from Montingelli et al. [26]. Water-baths were used to keep the reactors at a fixed mesophilic temperature of 38 ± 1 °C for the duration of a retention time of 21 days. A control sample of each inocula in double replication was used to determine the inoculum

contribution to the biogas formation, which has been then subtracted from the biogas co-digestion volume in order to determine the actual yields of the seaweed residues.

2.6. Chemical oxygen demand of extracted macroalgae leachates

The total chemical oxygen demand (tCOD) content is widely used to evaluate the amount of organic matter within water and wastewater. This parameter was used in this study to estimate the organic matter dissolved in the residue samples. The procedure for tCOD analysis was performed according to Hach Lange [27]. The procedure involved a Hach Lange standard kit (range 0–1500 mg L^{-1}, Düsseldorf, Germany) and a Hach Lange DR2000 spectrometer to read the tCOD concentrations in the samples.

2.7 Theoretical methane yields and anaerobic biodegradability index

Results from the elemental analysis, described in section 2.2., were used to derive the stoichiometric methane potential (SMP) of the seaweed species under investigation prior to and following the chemical extraction cascade using Buswell's equation in eq. 2 [28].

$$C_cH_hO_oN_nS_s + 1/4(4c - h - 2o + 3n + 2s)H_2O = 1/8(4c + h - 2o - 3n - 2s)CH_4 + 1/8(4c - h + 2o + 3n + 2s)CO_2 + nNH_3 + sH_2 \quad \text{(eq. 2)}$$

A biodegradability index (BI) in eq. 3 was used to estimate the digestion efficiency via biochemical methane potential (BMP) essays. The BMP was calculated as % of the SMP yield from eq. 2 of the extracted feedstock achieved at the end of the digestion period.

$$\mathbf{BI}\ \% = \frac{\mathbf{SMP - BMP}}{\mathbf{SMP}} \times \mathbf{100} \quad \text{(eq. 3)}$$

3. Results and discussion:

3.1 Effect of seasonality and harvest location on organics composition

Results of the proximate analysis on the freshly collected seaweed biomass are reported in Table 1, while results for the extracted residues are shown in Table 2.

		VS% of TS						TS%					
		May	Jun	Sep	Oct	Nov	Jan	May	Jun	Sep	Oct	Nov	Jan
East	FS*		82 (0.17)		77 (0.08)	76 (0.23)	70 (0.11)		26 (0.01)		24 (0.14)	18 (0.01)	21 (0.31)
	FV*		74 (0.11)		71 (0.85)	76 (0.01)	66 (0.23)		19 (0.13)		26 (0.02)	22 (0.06)	25 (0.16)
	AN*		74 (0.43)		72 (0.07)	72 (0.17)	66 (0.01)		26 (0.18)		28 (0.04)	25 (0.24)	29 (0.27)
	LD*		76 (0.20)		71 (0.10)	75 (0.18)	66 (0.09)		21 (0.22)		16 (0.13)	14 (0.21)	14 (0.17)
	LS*		73 (0.13)		70 (0.41)	78 (0.00)	72 (0.20)		16 (0.14)		18 (0.21)	19 (0.01)	17 (0.11)
West	FS*	79 (0.03)		73 (0.47)		77 (0.02)	70 (0.45)	19 (0.19)		29 (0.11)		22 (0.12)	18 (0.02)
	FV*	73 (0.14)		68 (0.44)		76 (0.41)	76 (0.26)	20 (0.21)		28 (0.07)		24 (0.23)	21 (0.06)
	AN*	73 (0.25)		76 (0.05)		80 (0.15)	75 (0.19)	28 (0.15)		34 (0.13)		30 (0.16)	23 (0.08)
	LD*	73 (0.55)		71 (0.26)		70 (0.05)	66 (0.01)	11 (0.09)		18 (0.16)		15 (0.05)	13 (0.21)
	LS*	70 (0.34)		81 (0.09)		80 (0.04)	72 (0.18)	13 (0.10)		24 (0.22)		26 (0.11)	16 (0.13)

(*): FS=*Fucus serratus*, FV=*Fucus vesiculosus*, AN=*Ascophyllum nodosum*, LD=*Laminaria digitata*, LS=*Laminaria saccharina*.

Table 1. Dry matter and organic fraction in fresh un-extracted seaweed samples.

		VS% of TS						TS%					
		May	Jun	Sep	Oct	Nov	Jan	May	Jun	Sep	Oct	Nov	Jan
East	FS*		71 (0.12)		76 (0.13)	78 (0.01)	77 (0.01)		16 (0.02)		25 (0.23)	28 (0.27)	22 (0.11)
	FV*		77 (0.05)		77 (0.07)	72 (0.22)	76 (0.05)		21 (0.17)		31 (0.03)	26 (0.13)	24 (0.16)
	AN*		78 (0.04)		73 (0.08)	68 (0.12)	77 (0.03)		19 (0.04)		25 (0.11)	26 (0.18)	23 (0.21)
	LD*		71 (0.11)		74 (0.01)	74 (0.04)	68 (1.14)		14 (0.16)		21 (0.31)	23 (0.01)	15 (0.26)
	LS*		69 (0.16)		74 (0.07)	68 (0.08)	75 (0.16)		11 (0.06)		20 (0.24)	22 (0.08)	17 (0.22)
West	FS*	73 (0.09)		77 (0.04)		70 (0.80)	77 (0.17)	25 (0.14)		29 (0.16)		14 (0.22)	24 (0.13)
	FV*	77 (0.09)		75 (0.56)		74 (2.67)	75 (0.35)	30 (0.19)		29 (0.15)		13 (0.29)	24 (0.09)
	AN*	64 (0.04)		71 (0.01)		79 (0.02)	78 (0.08)	33 (0.22)		26 (0.05)		15 (0.04)	28 (0.03)
	LD*	51 (0.35)		72 (0.02)		78 (0.92)	78 (0.04)	16 (0.25)		21 (0.18)		10 (0.07)	21 (0.10)
	LS*	70 (0.01)		72 (0.00)		71 (0.08)	73 (0.15)	15 (0.21)		20 (0.10)		12 (0.15)	16 (0.17)

(*): FS=*Fucus serratus*, FV=*Fucus vesiculosus*, AN=*Ascophyllum nodosum*, LD=*Laminaria digitata*, LS=*Laminaria saccharina*.

Table 2 Dry matter and organic fraction in extracted residues

The proximate compositions of fresh samples generally reflect the range values found in the studies conducted by Allen et al. [5] and Edward et al. [29] on composition of brown seaweeds for AD in August. The first was conducted in the South coast of Ireland, while the second study analysed feedstock collected on the East coast of UK near Newcastle. These sites are bathed by the same geographical waters (less than 600 km from each other), i.e. the Irish Sea and the Northern Sea. Comparing results, however, it can be observed that the organic fraction in the dry matter heavily depends on location of harvesting within the same season. For example, Allen et al. has identified a VS% content of about 85% in FS collected in Southern Ireland in the summer. This value is very close to this study's findings for the same species on East Ireland (82%) however, on the West coast this decreases to an estimated 76% with a drop of about 9% in organics content. Similarly, comparing VS% in LD with the studies by Allen et al. and Edward et al. in the late summer with this study's findings, three different values can be noted at different sites of harvesting: 73% South Ireland [5], 72% East UK [29] and around 71% West Ireland. Another example can be identified in the study by Tabassum et al. [12], which investigated the seasonal composition and biomethane production from AN. In November, the VS% of AN changes depending on geographical location of harvesting, e.g. 82% [12], 72% and 80% in the South, East and West of Ireland respectively. Again in November, another study on LD's seasonal characterisation for biomethane production by Tabassum et al. [30], reports the organics fraction in biomass harvest from the South of Ireland at 75%. This value is maintained in harvest from the East, while a drop of 5% is observed feedstock collected in the West. Changes in VS% (of TS) can therefore be considered as location's dependant, and such behaviour is exhibited by all species to different extents according to seasons. This is also reflected in the macroalgal residues' composition. In Table 2, it can be observed that the proximate composition of each species presents changes from minor to significant in organics content depending on harvest location. In November for instance, FV's variation among locations is about 2%, while AN's can vary by 11%. Large variations in organics are of tremendous significance to the rates of biofuel conversion that can be expected, as actual methane yields are positively related to the amount of VS in biomass.

In relation to seasons, a study by Marinho-Soriano et al. [31], reports a positive correlation between carbohydrates content and temperature, while another study relates higher light intensity to increased production of polysaccharides [32]. Also, a study by Black [33] reports

that sugars concentration is high in the summer period for brown seaweeds. Therefore, in freshly harvested feedstocks the highest methane potential is expected in the warmer seasons due to the highest amount of VS content, see also Table 1. This has been confirmed by a study on seasonal biomethane yields from LD, where July is considered the best month for harvest [34]. However, this cannot be systematically the case as the concentration of inhibitors, such as polyphenols and salt accumulation, has been found to affect the actual conversion to methane [12].

From Table 2, a seasonal variation in organics can also be observed in the macroalgal residues. However, the final composition of the residues cannot solely be attributed to the starting composition, which is certainly also dependent on seaweed species, harvesting location and extraction procedures, including possible interactions between such factors. We suggest possible explanations for gain or loss of VS% found in the residues involve factors such as formations of pH gradients during the process, different plant's membrane permeability and residual vitality of the plant, which determines the stock's susceptibility to the extractions. Furthermore, since feedstocks were extracted all at once to replicate the industrial process, partial migration and/or retention of organics from the reagent solutions cannot be excluded. Overall it can be stated that highest values of VS are more recurrent in the colder months for all species.

3.2 Effect of bioproducts extraction cascade on suitability for AD

Outcomes of the ultimate analysis on fresh un-extracted and extracted biomass species are reported in Table 3, showing the weight in % of C, H, N, S and O in the samples against each species across harvest periods. When missing, values have been interpolated between the previous and the following period recorded, assuming linear correlation. The combined effect of seasonal variation, harvest site and extraction procedures on the C:N ratio and residues' tCOD concentrations is reported in Table 4.

		Fresh samples										Extracted residues									
	Period	East					West					East					West				
		FS [h]	**FV** [h]	**AN** [h]	**LD** [h]	**LS** [h]	**FS** [h]	**FV** [h]	**AN** [h]	**LD** [h]	**LS** [h]	**FS** [h]	**FV** [h]	**AN** [h]	**LD** [h]	**LS** [h]	**FS** [h]	**FV** [h]	**AN** [h]	**LD** [h]	**LS** [h]
%C [a,c]	MAY						39.2	37.2	37.8	40.1	36.0						40.4	40.4	37.4	29.8	31.4
	JUNE	46.8	39.2	39.3	39.1	39.0	40.6*	38.3*	39.8*	38.1*	37.4*	37.1	39.5	40.8	38.9	37.1	40.7*	40.0*	38.4*	33.0*	34.0*
	SEPT	41.8*	39.2*	38.6*	36.6*	37.1*	42.1	39.5	41.9	36.1	38.7	38.7*	40.1*	39.7*	37.9*	37.7*	41.1	39.6	39.4	36.2	36.7
	OCT	38.4	38.5	37.5	32.5	32.2	39.6*	38.7*	40.7*	34.0*	37.5*	40.3	40.6	38.5	36.8	38.4	39.4*	39.8*	40.9*	36.7*	36.7*
	NOV	36.8	39.1	37.8	34.2	35.2	37.2	38.0	39.6	31.8	36.3	40.8	38.3	36.6	37.5	35.5	37.7	40.0	42.4	37.2	36.6
	JAN	35.2	35.5	36.5	29.8	32.5	36.1	37.8	39.3	29.9	32.8	40.7	40.1	40.6	36.5	38.4	42.9	40.2	44.3	41.4	40.5
%H [a,d]	MAY						5.3	4.5	5.2	4.4	4.5						4.5	4.9	5.4	3.1	4.2
	JUNE	5.7	1.6	4.5	1.8	4.9	5.0*	4.5*	5.1*	4.3*	4.6*	3.7	4.5	4.2	4.5	4.0	4.6*	4.7*	5.0*	3.9*	4.2*
	SEPT	5.3*	3.2*	4.7*	3.3*	4.8*	4.7	4.4	5.0	4.2	4.8	4.4*	4.7*	4.5*	4.7*	4.5*	4.8	4.6	4.6	4.8	4.3
	OCT	5.0	4.8	4.9	4.7	4.6	4.9*	4.5*	5.0*	4.5*	4.9*	5.0	5.0	4.7	4.9	5.1	4.7*	4.9*	5.0*	5.3*	4.5*
	NOV	5.3	4.8	4.6	4.6	5.8	5.0	4.6	5.1	4.7	5.0	4.6	4.2	4.3	6.1	5.9	4.7	5.1	5.4	5.8	4.8
	JAN	4.4	4.4	4.5	4.7	4.3	4.2	4.4	4.8	4.1	4.1	4.5	4.7	4.6	4.3	4.6	4.6	4.7	4.8	4.8	4.7
%N [a,e]	MAY						2.1	2.2	1.5	3.0	2.7						1.9	1.8	1.3	1.4	2.3
	JUNE	2.3	4.6	2.0	5.3	2.0	1.7*	1.6*	1.1*	2.2*	1.8*	2.2	1.6	1.8	1.8	1.7	1.6*	1.3*	1.0*	1.2*	1.5*
	SEPT	2.0*	3.1*	1.6*	3.5*	2.1*	1.3	0.9	0.8	1.3	0.8	1.9*	1.6*	1.5*	1.7*	1.7*	1.4	0.9	0.7	0.9	0.7
	OCT	1.7	1.6	1.3	1.7	2.3	1.3*	1.2*	0.8*	1.4*	1.1*	1.6	1.7	1.2	1.6	1.7	1.2*	1.2*	0.7*	1.2*	1.0*
	NOV	2.0	2.2	1.6	1.9	2.7	1.3	1.5	0.8	1.4	1.3	1.5	1.9	1.3	1.4	1.4	1.1	1.6	0.7	1.5	1.3
	JAN	1.9	2.3	1.8	2.1	3.2	2.1	2.7	1.6	2.4	3.0	1.8	2.0	1.3	1.7	1.7	2.0	1.7	1.2	2.0	1.9
%S [a,f]	MAY						1.1	2.3	1.5	1.1	0.8						0.8	2.9	2.5	0.3	0.8
	JUNE	1.4	4.2	1.7	1.1	0.8	1.5*	1.9*	1.7*	1.1*	0.8*	1.2	2.3	2.1	0.6	0.4	1.1*	2.1*	2.0*	0.5*	0.6*
	SEPT	1.6*	3.2*	1.9*	1.5*	1.0*	1.9	1.6	1.8	1.0	0.8	1.2*	2.0*	1.9*	0.8*	0.5*	1.4	1.3	1.6	0.7	0.5
	OCT	1.9	2.3	2.2	2.0	1.2	1.7*	1.6*	1.6*	1.2*	0.8*	1.3	1.8	1.7	1.0	0.7	1.3*	1.6*	1.7*	0.6*	0.5*
	NOV	1.0	3.6	1.9	0.5	1.0	1.4	1.6	1.4	1.4	0.9	0.9	1.0	0.9	0.4	0.3	1.3	1.9	1.7	0.5	0.6

	JAN	1.3	1.8	1.2	0.5	0.9	1.7	1.5	1.9	1.2	1.0	0.9	1.2	0.9	0.7	0.5	1.6	2.1	0.9	1.4	0.4
%O[a,b,g]	MAY						31.9	27.0	27.0	24.4	26.4						0.8	2.9	17.3	0.3	0.8
	JUNE	25.5	24.6	27.0	28.5	26.4	27.6*	24.3*	26.7*	26.6*	31.3*	27.3	28.9	29.4	25.0	25.9	1.1*	2.1*	9.4*	0.5*	0.6*
	SEPT	27.8*	24.4*	26.7*	29.4*	28.2*	23.3	21.7	26.5	28.8	36.3	14.3*	15.3*	15.5*	13.0*	13.3*	1.4	1.3	1.6	0.7	0.5
	OCT	30.1	24.2	26.4	30.3	30.0	27.7*	25.8*	29.8*	29.9*	36.3*	1.3	1.8	1.7	1.0	0.7	1.3*	1.6*	1.7*	0.6*	0.5*
	NOV	31.2	26.7	26.4	34.1	33.2	32.1	29.8	33.2	30.9	36.3	0.9	1.0	0.9	0.4	0.3	1.3	1.9	1.7	0.5	0.6
	JAN	27.3	22.2	22.2	29.0	31.1	25.9	29.8	27.8	28.0	31.3	0.9	1.2	0.9	0.7	0.5	1.6	2.1	0.9	1.4	0.4

[a] Molecular weight: C=12.01, H=1.01, N=14.00, S=32.07, O=15.99.
[b] Oxygen content was calculated by difference according to (eq.1).
[c] standard deviation range across measurements 0.00-0.30.
[d] standard deviation range across measurements 0.01-0.13.
[e] standard deviation range across measurements 0.00-0.07.
[f] standard deviation range across measurements 0.00-0.59.
[g] standard deviation range across measurements 0.00-0.65.
[h] FS=*Fucus serratus*, FV=*Fucus vesiculosus*, AN=*Ascophyllum nodosum*, LD=*Laminaria digitata*, LS=*Laminaria saccharina*.
[*] estimated averaged values among periods.

Table 3 Elemental composition in % of dry matter (TS) of the seaweed sp. samples by season and harvest location

			MAY	JUNE	SEPT	OCT	NOV	JAN
FS*	East	C:N initial		20.3		22.3	18.1	18.5
		C:N Residual		16.9		24.4	27.0	22.5
		tCOD Res [g/l]		12.7±0.001		29.3±0.003	39.5±0.001	25.1±0.001
	West	C:N initial	19.0		32.7		29.7	16.9
		C:N Residual	21.7		29.4		25.3	21.2
		tCOD Res [g/l]	22.1±0.002		22.6±0.001		20.9±0.002	18.5±0.001
FV*	East	C:N initial		8.5		23.4	17.8	15.6
		C:N Residual		24.7		24.0	20.7	19.6
		tCOD Res [g/l]		16.6±0.002		27.4±0.004	20.4±0.001	9.0±0.002
	West	C:N initial	17.2		41.8		25.3	14.2
		C:N Residual	22.3		44.7		25.3	24.2
		tCOD Res [g/l]	23.2±0.007		18.6±0.001		15.8±0.003	23.3±0.002
AN*	East	C:N initial		19.7		29.2	23.9	20.6
		C:N Residual		24.7		33.2	27.5	31.7
		tCOD Res [g/l]		17.1±0.003		18.7±0.003	11.2±0.001	18.2±0.001
	West	C:N initial	25.9		51.1		49.5	24.3
		C:N Residual	29.8		60.2		59.7	36.0
		tCOD Res [g/l]	27.4±0.006		15.9±0.0011		17.5±0.004	16.1±0.001
LD*	East	C:N initial		7.4		18.8	18.2	14.0
		C:N Residual		21.6		22.6	25.9	22.1
		tCOD Res [g/l]		32.4±0.001		51.0±0.001	35.5±0.001	18.2±0.001
	West	C:N initial	13.2		27.5		22.2	12.4
		C:N Residual	23.2		40.5		23.9	21.1
		tCOD Res [g/l]	18.5±0.001		28.2±0.008		34.9±0.003	24.6±0.001
LS*	East	C:N initial		19.5		14.3	12.9	28.1
		C:N Residual		21.8		22.1	24.5	22.9
		tCOD Res [g/l]		20.7±0.001		35.3±0.002	28.1±0.001	17.4±0.001
	West	C:N initial	13.2		47.2		27.7	10.9
		C:N Residual	13.5		53.6		27.8	21.9
		tCOD Res [g/l]	14.8±0.008		21.4±0.001		33.3±0.001	25.4±0.000

(*): FS=*Fucus serratus*, FV=*Fucus vesiculosus*, AN=*Ascophyllum nodosum*, LD=*Laminaria digitata*, LS=*Laminaria saccharina*.

Table 4. Seasonal and geographical variation of C:N ratio and tCOD measurements.

The extraction procedure has generally caused an increase in C:N ratio across the samples, which in most cases means an improved suitability for AD. For example, FS harvested on the East coast naturally presents a value of 8.5 which is too low for a stable digestion. After extraction this increases to 24.7, which instead is in the range of what the literature [35]

suggests to promote stable yields and avoid inhibition. This would indicate that seaweed residues following alike-extraction procedures would be more suitable for biogas conversion than fresh or drift seaweed substrates alone. In the actual anaerobic fermentation trials in co-digestion, the C:N parameter fell within the ideal range as adjusted by the carbon and nitrogen content of the sludges used as inocula.

The tCOD concentrations in Table 4 for the residues are above those obtained from seaweed leachate by Nkemka and Murto [36], which found a value of about 10 g L^{-1} in tCOD after removal of heavy metals. Montingelli et al. has investigated the biomethane potential of a mixture of brown seaweeds at three different harvesting periods [13] to estimate the benefits of mechanical pretreatments. In the compositional analysis of the untreated seaweed mix, which presented the highest values, the authors report soluble COD (sCOD) values up to 7.6 g L^{-1} in May, 12.43 g L^{-1} in November, and 12.80 g L^{-1} in March, these indicate higher values in tCOD. The values obtained in this research are more in line with the tests conducted by Gurung et al. [37] on fresh samples of seaweeds among other substrates. Fresh mixtures of green and brown algae resulted in a tCOD of 26 ± 0.1 g L^{-1} and 31 ± 0.1 g L^{-1} respectively. The results show a positive correlation between tCOD content and methane yield however, there appears to be an upper limit to the benefit of increasing organics in the digester, due to overload or inhibition by other toxic compounds. In our study higher concentrations indicate a significant amount of organic matter is present following the extractions and it is available to be converted to biogas via AD. The high values recorded in some species' leachates can also be the result of the organic solvents used, which could have been retained within the plant cells.

Table 5 shows the results of the SMP calculations described by eq. 2, section 2.7. It also reports the average seasonal value of the C:N ratio. This is higher than for fresh biomass (Table 3), which highlights an improvement towards a better digestibility for the residual samples (C:N>20). This makes the macroalgal residues a more flexible substrate for co-digestion with other biowaste, with the exception of AN and *Laminaria* spp. harvested in September and November on the West coast. These are too high in C:N (>30), refer to Table 4, and will therefore need to be balanced by co-digestion with nitrogen-rich substrates. As noted for variation in VS% in section 3.1, also a gain/loss effect in SMP can be observed if compared with the fresh feedstock.

The SMP values obtained for AN are in line with values found by Tabassum et al. [12], which calculated the potential methane yield variations according to seasons, while for the other species are in agreement with values found by Allen et al. [5] for all other species in the summer period. This demonstrates the extracted residues have great potential to be used as substrates in AD systems. From Table 5, the highest average SMP of the residues is provided by the *Laminaria* spp. (about 525 mL CH_4 gVS^{-1}) harvested on the East coast, while the most promising residual substrate on the West would be AN with an average of 567 mL CH_4 gVS^{-1}. Feedstock being equal, the site of harvest again can be seen affecting the seasonal SMP values for the fresh as well as the residues. For example, values from residues of FS in November differ by about 8%, favouring collection from the West rather than the East coast.

		SMP (fresh) [mL CH_4 gVS^{-1}]						SMP (residues) [mL CH_4 gVS^{-1}]					
		MAY	JUNE	SEPT	OCT	NOV	JAN	MAY	JUNE	SEPT	OCT	NOV	JAN
East	FS		601		465	469	471		473		518	490	501
	FV		352		523	464	519		493		503	496	498
	AN		494		502	497	547		485		509	531	500
	LD		361		419	413	436		550		481	555	526
	LS		363		425	447	398		514		516	575	493
West	FS	486		563		457	476	552		507		531	525
	FV	493		574		469	448	495		504		533	509
	AN	536		542		469	495	627		546		535	561
	LD	533		468		422	410	576		496		487	505
	LS	356		435		418	393	397		484		507	555
Average Seasonal C:N		17.7	15.1	40.1	21.6	24.5	17.5	22.1	21.9	45.7	25.3	28.8	24.3

FS=*Fucus serratus*, FV=*Fucus vesiculosus*, AN=*Ascophyllum nodosum*, LD=*Laminaria digitata*, LS=*Laminaria saccharina*.

Table 5 Stoichiometric methane potential (SMP) of fresh and extracted seaweeds by harvesting site

The use of *Laminaria* spp. harvested in the East of Ireland and AN in the West would therefore improve the biomethane rates achievable from the macroalgal residues. Theoretical yields calculated from the chemical composition of macroalgae using eq.2 can be high however, practical yields of biogas from the anaerobic digestion of seaweed are considerably below the theoretical maximum [38]. This appears to be due to hydrolysis of complex polysaccharides such as alginates, which is regarded as the rate limiting step for AD of seaweed [39]. As part of these polysaccharides has been partially removed by the bioproducts extraction procedure, higher biodegradability should be possible in the actual fermentation (BMP).

3.3 Effect of inoculum acclimatation on methane production

The final pH was measured at the end of every digestion period for all reactors and it was found ranging between 7.5 and 7.9. Therefore, in this study such parameter did not affect the digestion significantly. Results from the BMP trials set up as described in section 2.5 are presented in Table 6 and include the derived BI values in relation to each type of inoculum used. Practical yields from AD of fresh harvested seaweeds have been reported between 140 and 400 mL CH_4 gVS^{-1} [7, 40]. A study on *Sargassum* and *Gracilaria* spp. reported instead a greater yields range between 280 and 400 mL CH_4 gVS^{-1}, with bioconversion rates of 58-95% [41]. These values are in line for most methane yields obtained in this study from the macroalgal residues, demonstrating the high value of these as bioresource for energy generation. In particular, methane yields between 47-535 mL CH_4 gVS^{-1} are observed when co-digesting with sludge, while rates between 27-523 mL CH_4 gVS^{-1} result from co-digestion with acclimatised sludge. Bioconversion greater than 70% was achieved on the *Laminaria* spp. regardless of the harvest site as well as FS harvest from the West coast. Furthermore, the two *Laminaria* species also presented the highest average CH_4 production rate achieved with harvest from both locations, see average values in Table 6.

In particular, the best results were obtained from LD residue from the East coast with acclimatised sludge (499 mL CH_4 gVS^{-1}), while on the West coast they are achieved co-digesting LS residue with non-acclimatised sludge (504 mL CH_4 gVS^{-1}). Good performance of the inoculum could be caused by sugars being available in their simpler forms due to partial hydrolysis occurred as well as part of the alginic acid being lost for these species during the extraction. Also, a very interesting study by Sutherland et al. [39], investigated seven different microbial inocula and a mixture of these to test the efficiency at degrading *Laminaria hyperborea* seaweed and produce methane through AD. The authors conclude that higher bacterial charge in the inoculum should lead to higher methane yields due to acclimatation to digest phycocolloids, which can explain the higher rates for LD.

As expected from the SMP indicators, both *Laminaria* species resulted in the highest BMP yields however, AN harvest has been converted to only half of its potential, resulting in a BI of 0.53 in the spring. The actual CH_4 yield from fresh AN from Scandinavian waters, was also found at its maximum in May by Ometto et al. [42] however, this was extremely low (70-80 Nml/gVS_{add}) compared to our findings. This could be due to a significant difference found in proximate composition (>10%TS) and highly variable content in polyphenols for AN according to seasons which peaks in the spring [12]. Insoluble fibers and polyphenols are

known to be difficult to degrade and potentially inhibiting for AD [43]. Also, biodegradability of biomass can be affected by several other factors like crystalline structure, the extent of cellulosic polymers, the surface properties of biomass, the amount of lignin content, the presence of hemicellulosic materials and the strength of fibers [44]. Other relevant studies were conducted on factors affecting the microbial digestion of an industrial seaweed-based residue of AN post alginate extraction [45, 46]. The residue characterised by these showed an alkaline pH of the substrate prior to digestion and the authors identified that the digestibility was influenced by soluble/insoluble matter and inocula's metabolite inhibition, which are critical features for the digestion of the residues. They concluded that similar organic residues require a carefully chosen inoculum and a minimum initial insoluble content (65–70%) and/or a maximum soluble content (25-30%) of dry weight.

		BMP – Sludge [mL CH_4 gVS^{-1}]							BI – Sludge inoculum						BMP – Acclimatised Sludge [mL CH_4 gVS^{-1}]							BI – Acclimatised Sludge					
		MAY	JUNE	SEPT	OCT	NOV	JAN	Average	MAY	JUNE	SEPT	OCT	NOV	JAN	MAY	JUNE	SEPT	OCT	NOV	JAN	Average	MAY	JUNE	SEPT	OCT	NOV	JAN
East	FS*		248±11		317±14	293±17	143±13	250		0.52		0.61	0.60	0.28		63±18		246±18	283±10	226±64	205		0.13		0.47	0.58	0.45
	FV*		189±15		18±18	180±18	52±28	110		0.38		0.04	0.36	0.10		154±18		66±16	111±22	97±17	107		0.31		0.13	0.22	0.19
	AN*		209±9		172±19	106±12	173±29	165		0.43		0.34	0.20	0.35		167±29		142±20	181±27	256±21	187		0.34		0.28	0.34	0.51
	LD*		184±15		320±12	91±6	404±12	250		0.33		0.66	0.16	0.77		499±17		340±43	127±6	495±20	365		0.91		0.71	0.23	0.94
	LS*		285±12		471±15	218±24	243±32	304		0.55		0.91	0.38	0.49		88±16		433±16	255±35	385±28	290		0.17		0.84	0.44	0.78
West	FS*	279±19		129±18		416±21	125±27	223	0.51		0.25		0.78	0.24	363±21		161±19		271±16	198±14	248	0.66		0.32		0.51	0.38
	FV*	47±25		167±17		327±10	176±26	223	0.09		0.33		0.61	0.35	192±25		159±14		27±6	156±4	134	0.39		0.31		0.05	0.31
	AN*	334±32		96±15		340±24	92±23	176	0.53		0.18		0.64	0.16	144±58		113±19		123±22	141±29	130	0.23		0.21		0.23	0.25
	LD*	187±9		368±7		132±41	147±11	216	0.33		0.74		0.27	0.29	523±17		304±18		158±21	240±35	306	0.91		0.61		0.32	0.47
	LS*	320±11		177±9		504±11	535±5	405	0.81		0.37		0.99	0.97	236±25		110±13		312±19	436±23	273	0.59		0.23		0.61	0.79

(*): FS=*Fucus serratus*, FV=*Fucus vesiculosus*, AN=*Ascophyllum nodosum*, LD=*Laminaria digitata*, LS=*Laminaria saccharina*.

Table 6 Biochemical methane potential (BMP) and biodegradability indices (BI) obtained with and without acclimatation

Seasonal BIs are plotted for each species in Figures 1-5 against tCOD [10^{-2} g L^{-1}] concentrations and ash-to-volatile (A:V) ratio in % within the samples, to help evaluate the interactions between composition and digestion performance. It can be noticed that in many instances the BIs' trend follows the distribution of the tCOD parameter, e,g. Figure 3(a). In other cases, these appear to follow the trend exhibited by the result of both indicators' influence, e.g. Figure 3(b). From Figures 2(b)) and 5(b), it can be observed that the A:V ratio appears fairly stable with low variations, hence this is assumed not to be significant on the final methane yield. Higher values of A:V ratio in the sample resulted in reduced BI due to higher insoluble matter, particularly when coupled with low tCOD concentrations in the reactor. This is particularly evident in Figure 3(a) and these conditions are responsible for the lowest yield in November.

Higher BIs are instead observed in presence of lower A:V ratio and higher tCOD concentrations. This can be seen clearly for FS in Figure 1(a), AN in Figure 3(b) and LD from both harvest sites, Figure 5. However, in the case of LD such parameters' combination has resulted in the lowest methane conversion achieved across the digestion periods. In November, when A:V ratio is at its minimum and tCOD is high, the final BI is below 0.32; refer to Figure 4(a) and (b). This appears to be due to an overload of organics in the reactors. Another study by Tabassum et al. [30], reports seasonal BI rates from fresh LD between 0.44 to 0.72 when seeding with dairy slurry, grass silage and seaweed, with biodegradation peaks achieved in the summer. In this investigation, the best BI values for LD were instead achieved in the spring with values above 0.9 with acclimatised sludge, producing about 34% extra methane compared to Tabassum et al.'s best results. In January high degradation was achieved (as high as May) with acclimatised inoculum, despite an increase in ash content for this period. This exhibits high tolerance for salts and other insoluble fibers during digestion.

To summarise the outcomes of the BMP trials, on an annual basis acclimatation has been found effective at improving yields from AN and LD residues harvested in the East of Ireland, with increased average yields of 12% and 32% respectively over non-acclimatised seeding. For harvest collected instead on the West coast, acclimatation is recommendable when digesting FS and LD residues, with an advantage of 10% and 30% extra methane production. Benefits of inoculum acclimatation are species dependant as well as composition being affected by harvest location. Co-seeding with a portion of acclimatised inoculum could be extremely beneficial to digest residues that performed closely in BMP values with different inocula, like for example FS harvested in the West (Figure 1(b)). In this case, the

addition of acclimatised inoculum will increase the BI by more than 20% on an annual basis, which will generate generous extra methane yields. Another study by Xia et al. [47] identified a change in process design via a two-stage digestion of marine feedstock can improve the quality of the biogas produced, as opposed to a single stage fermentation. Two-stage digestion has also been found to be increasing the methane production from AN residues after alginate extraction with yields of 237 mL CH_4 gVS^{-1} [48]. Further optimisations to improve biodegradability and increase methane production from seaweeds involve particle size reduction to maximise surface area available to enzymatic action. Previous published work by Tedesco et al. [49-51] has regarded mechanical comminution of fresh macroalgae with final CH_4 rates up to 53% higher than untreated biomass, particularly from *Laminaria* spp., when 80% of the particles were <1.6 mm^2. However, since mechanical size reduction is costly, the viability of integrating a pretreatment step will need to be assessed on a case by case basis. Similar work on pretreatment was conducted by Rodriguez et al. [52] on freshly harvested seaweed *Pelvetia canaliculata* in a multi-objective optimization using a response surface methodology with a composite central design. Inoculum-to-substrate (ISR) ratio was varied in addition to pretreatment time, where digested sludge was used for seeding. The best results were achieved with the highest ratio (1:0.3), meaning higher inoculum shares would lead to an improved digestion of fresh seaweed. We believe this to be the case also for the macroalgal waste streams analysed in this study, as generally there is a positive correlation between greater BIs (%) and higher bacterial charge in the reactor.

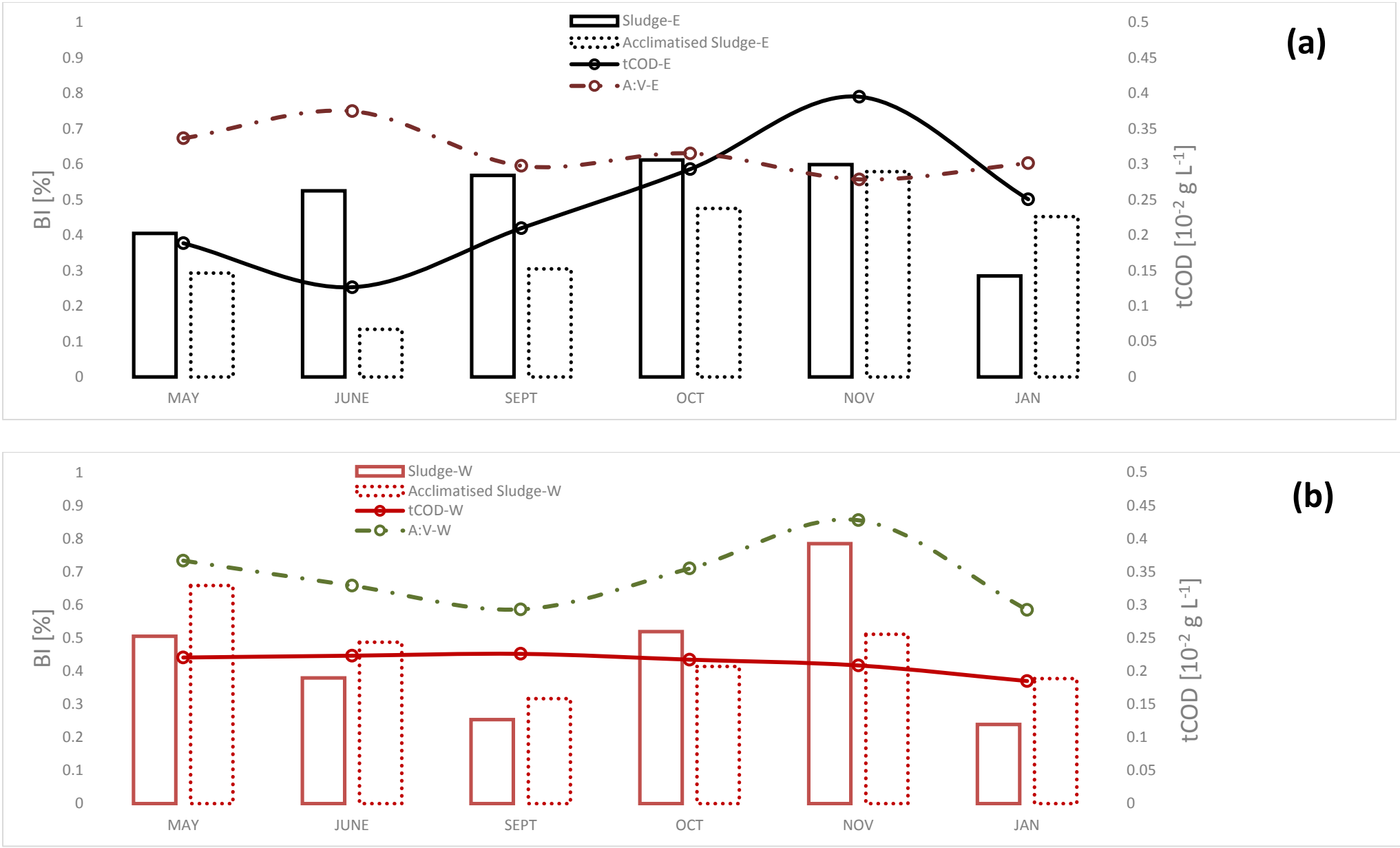

Figure 1 Biodegradability index (BI) variation of Fucus serratus in relation to tCOD content and A:V ration on samples harvested in the (a) East and (b) West coast of Ireland

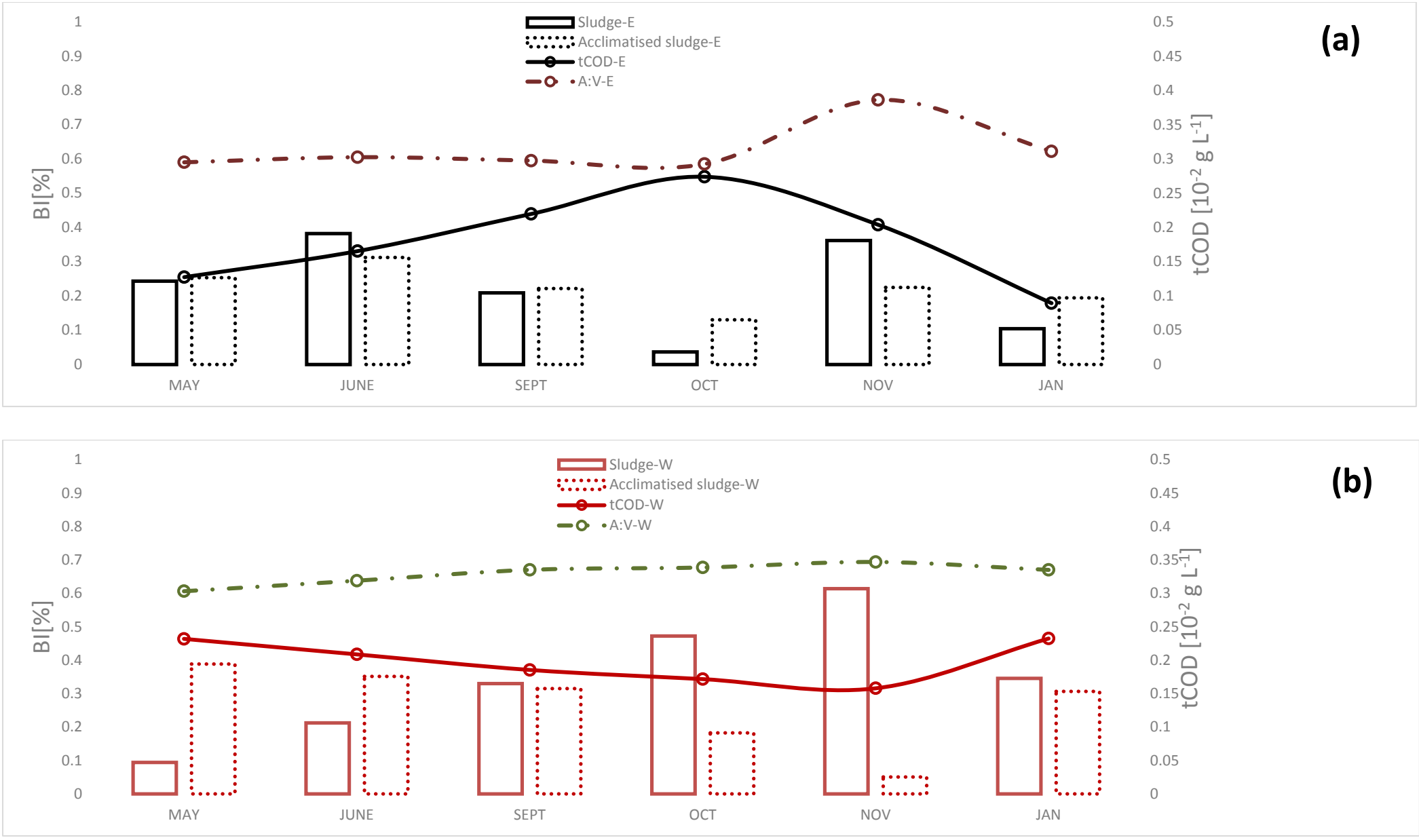

Figure 2 Biodegradability index (BI) variation of Fucus vesiculosus in relation to tCOD content and A:V ration on samples harvested in the (a) East and (b) West coast of Ireland

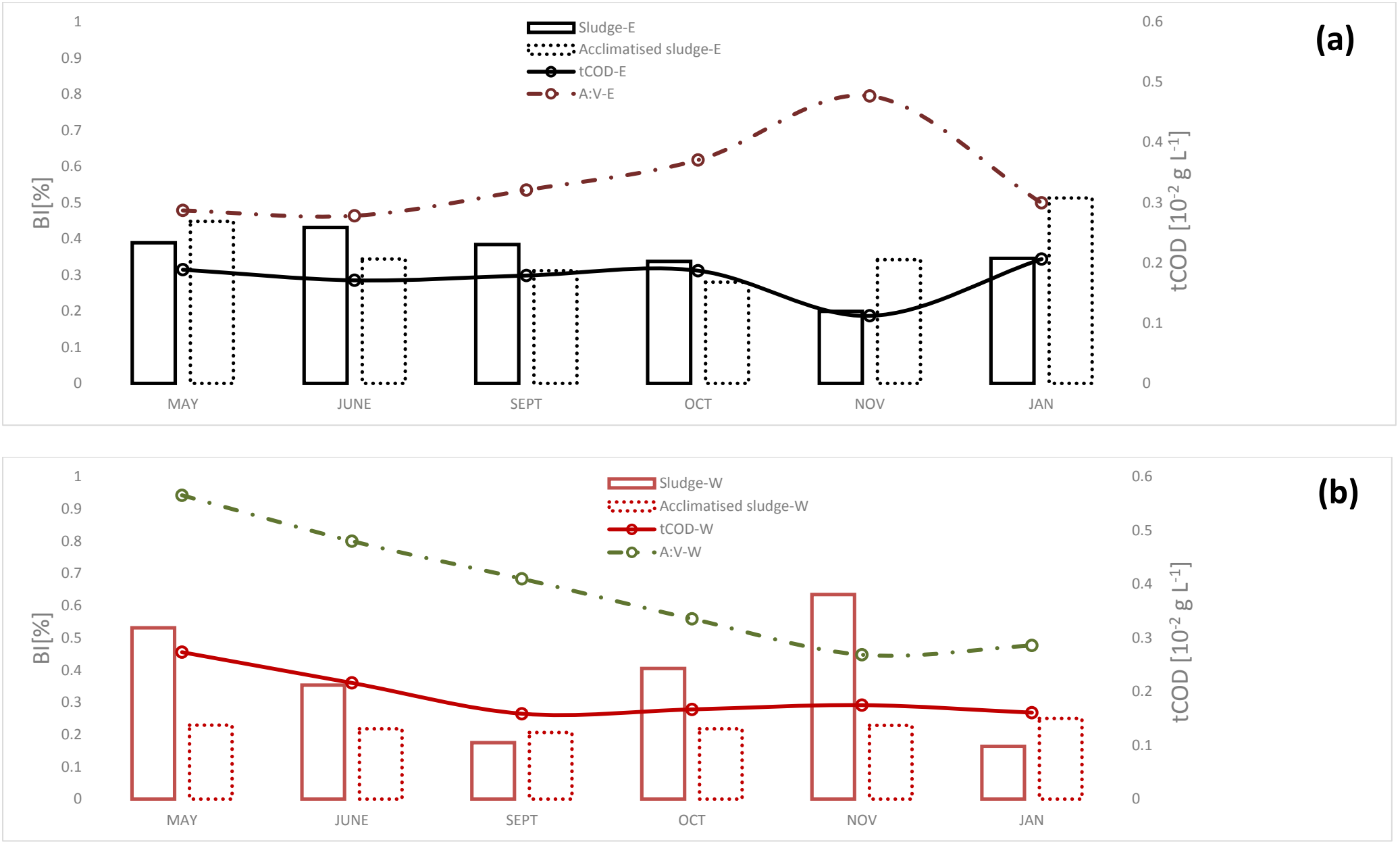

Figure 3 Biodegradability index (BI) variation of Ascophyllum nodosum in relation to tCOD content and A:V ration on samples harvested in the (a) East and (b) West coast of Ireland

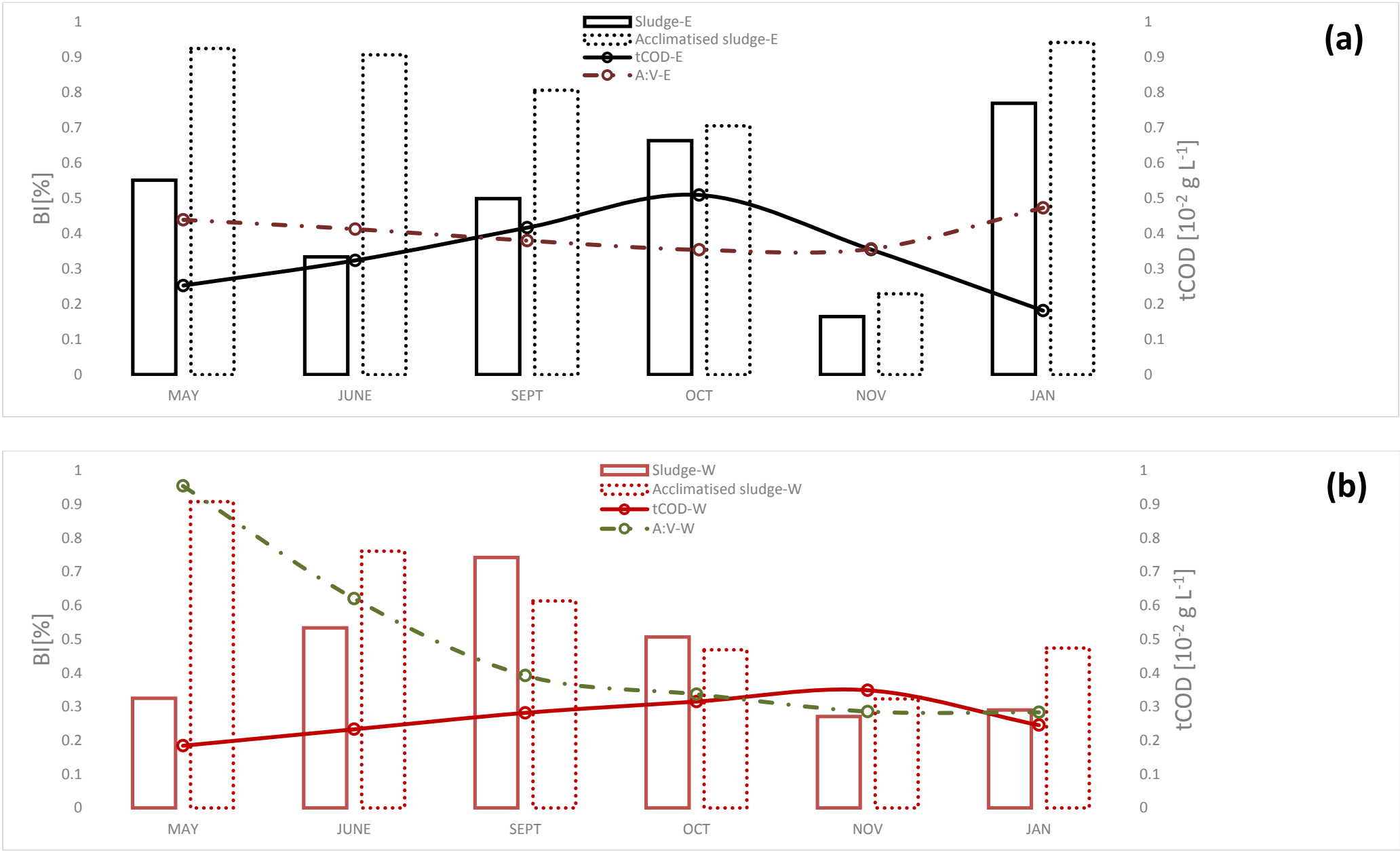

Figure 4 Biodegradability index (BI) variation of Laminaria digitata in relation to tCOD content and A:V ration on samples harvested in the (a) East and (b) West coast of Ireland

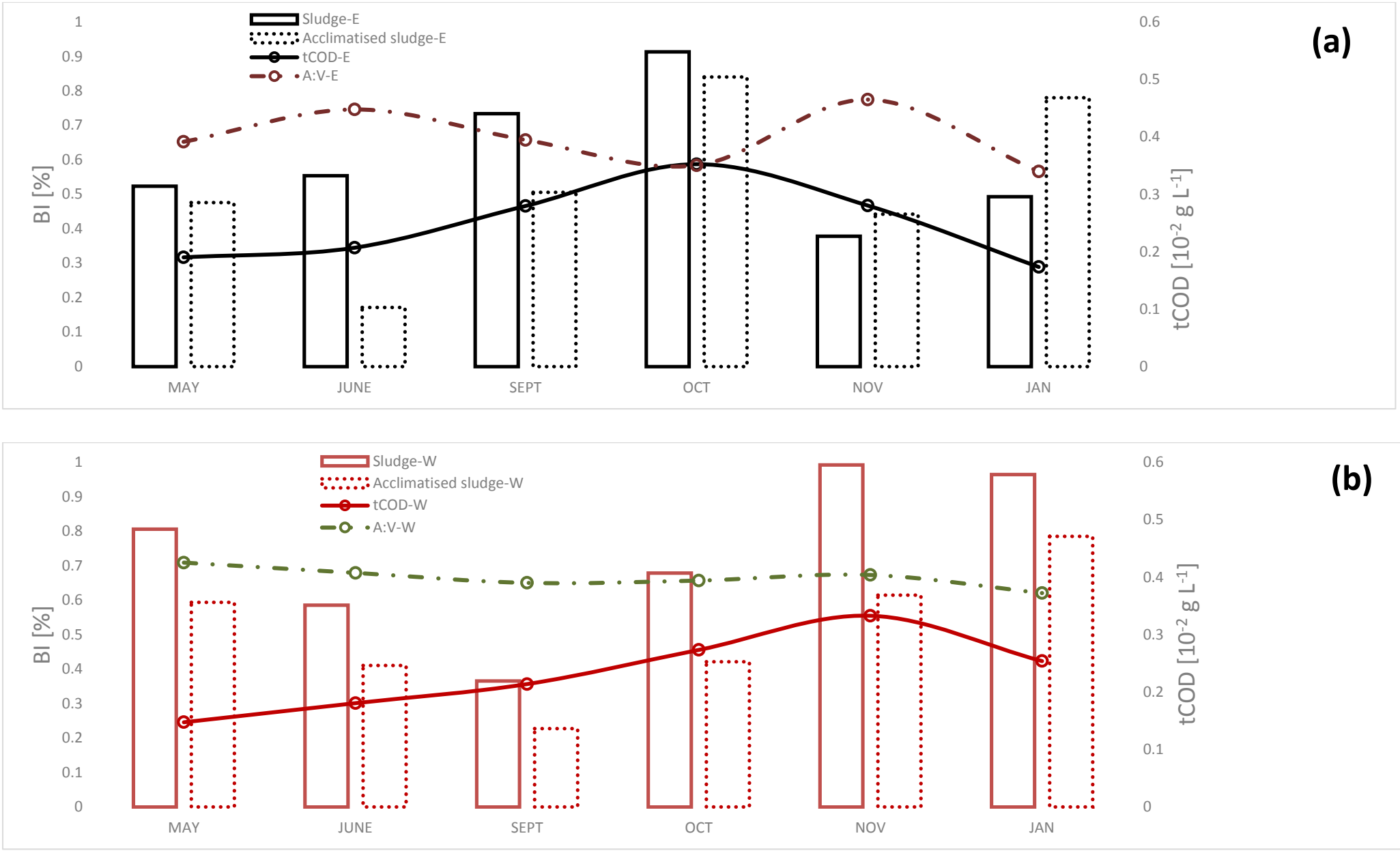

Figure 5 Biodegradability index (BI) variation of Laminaria saccharina in relation to tCOD content and A:V ration on samples harvested in the (a) East and (b) West coast of Ireland

4. Conclusion:

The potential of macroalgal biorefinery to produce bioenergy in the final step of the bioresource valorisation's chain is substantially unexploited. This study attempts to evaluate the feasibility of fermenting waste solids from seaweed processing plants to generate biogas on site. Seaweed residues from five spp. harvested from the East and West coastlines of Ireland were characterised and studied for anaerobic digestion. Factors examined were seasonal biochemical variation in relation to bioproducts extraction, which significantly affected the change in fermentable solids. Inoculum acclimatation was also tested to enhance the reactors performance. The results showed that acclimatation of inoculum is recommended for digestion of *Ascophyllum nodosum* harvested from the East coast, *Fucus serratus* from the West coast and generally for *Laminaria digitata*, with overall enhanced methane yields between 10-30% on an annual basis.

Important correlations were identified for seasonal biodegradability rates, whose trends were found to follow the distribution of the tCOD and/or the ash:volatile-solids parameter, depending on species and geographical harvest location. High average methane yields on an annual basis are estimated between 107-405 mL gVS^{-1} from the residues, demonstrating the worthiness of anaerobic digestion for macroalgal waste streams in this integrated biorefinery configuration. The use of organic solvents at ambient temperature for the extraction cascade allows for production of bioproducts and simultaneously enriches the residual substrates, improving suitability for biogas production. This would aid towards an enhanced efficiency of commercial plants through maximised exploitation of the seaweed resource.

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